Impact of Dietary Diversification on the Prognostic Inflammatory and Nutritional Index in School-Age Children in the Nawa Region (Côte d'Ivoire)

Allico Mousso Jean Maurel[1,2], Agbo Adouko Edith[3,4,*], Séri Kipré Laurent[1,2], Boyvin Lydie[1,2], Kouamé Christophe[4], Yapi Houphouët Félix[1] and Djaman Allico Joseph[1,2]

[1]*Biology and Health Laboratory, Félix Houphouët-Boigny University, 01 BP V34 Abidjan 01, Côte d'Ivoire*

[2]*Department of Medical and Fundamental Biochemistry, Institut Pasteur of Côte d'Ivoire (IPCI), 01 BP 490, Abidjan 01, Côte d'Ivoire*

[3]*Food Science and Technology Department, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire*

[4]*International Center for Research Agroforestry (ICRAF), Abidjan, 08 BP 2823 Abidjan 08, Côte d'Ivoire*

Abstract: The objective of this work was to study the impact of food diversification based on sweet potato, soybean, and cowpea on the prognostic inflammatory and nutritional index (PINI) in school-aged children in the Nawa region. This study took place from October 2017 to May 2018 among 240 pupils aged 6 to 12, divided into four groups of 60. Four types of meals were proposed: rice with tomato soup and fish (group 1), sweet potato porridge enriched with green soybeans (group 2), sweet potato porridge enriched with white cowpea (group 3), or sweet potato porridge accompanied by white cowpea with green soybeans (group 4). There were three blood samples: before eating meals (phase 0), the end of the first trimester (phase 1), and the end of the second trimester (phase 2). Blood assay for C-reactive protein (CRP), orosomucoid, albumin, and prealbumin was performed using COBAS c311 analyzer. PINI was calculated. Groups 3 and 4 showed a slight increase in albumin values (42.24 ± 0.95 g/L and 41.51 ± 1.71 g/L, respectively) compared to group 1. CRP decreased from phase 1 for group 1 (2.06 ± 0.26 mg/L) and group 4 (2.38 ± 0.36 mg/L). Orosomucoid increased insignificantly ($p > 0.05$) in group 3 (0.74 ± 0.04 g/L) and group 4 (0.71 ± 0.04 g/L). PINI was reduced by 0.37 (group 1), 0.36 (group 2), 0.46 (group 3) and 0.44 (group 4). Food diversification based on sweet potato and white cowpea has a positive impact on PINI in more than 80% of pupils.

Keywords: Côte d'Ivoire, cowpea, sweet potato, inflammatory and nutritional proteins, soybeans.

1. INTRODUCTION

In Côte d'Ivoire, malnutrition is one of the public health problems. In fact, in 2010, the prevalence of malnutrition among school children was 18.36 % in the Nawa region [1]. It has an impact on children's ability to learn and succeed [2]. In order to combat this situation, Agbo *et al*. [3] suggested dietary diversification for school-age children. According to the US Department of Agriculture and the US Center of Nutrition (1992), dietary diversification means "eat a variety of foods", on the premise that consuming a wide variety of foods will ensure an adequate intake of essential nutrients and, in turn, will lead to a better-quality diet and optimal health outcomes. In addition, the 2015-2020 US Dietary Guidelines recommend choosing a variety of nutrient-dense foods across all food groups and within all food groups, with particular emphasis on variety in vegetables and protein sources [4]. For the nutritional management of malnutrition based on the optimal use of locally available nutrient-rich foods to improve the nutritional status of children and prevent malnutrition [5], Agbo *et al*. [3] focused on nutrient-rich foods such as sweet potatoes, soybeans, and cowpeas. Indeed, the sweet potato (*Ipomoea batatas* Lam.) is energetic and is rich in vitamins and minerals (mainly potassium), dietary fiber, and proteins [6]. As for soybean (*Glycine max*), it is classified in the oil-protein category thanks to the richness of its seeds in macronutrients (proteins, lipids, and carbohydrates), micronutrients (minerals, vitamins), and secondary metabolites [7]. In addition, cowpea (*Vigna unguiculata* L. Walp.) represents an important source of protein for some populations [8].

Malnutrition causes a decrease in nutritional protein while it elevates inflammatory proteins [9]. Since the plasma concentrations of so-called nutritional proteins are influenced by inflammatory syndrome, Ingenbleek and Carpentier [10] proposed to correct the fluctuations of these proteins by variations in more specific proteins of an inflammatory syndrome such as C-reactive protein (CRP) and orosomucoid. In 1985, they proposed an index, called the prognostic inflammatory and nutritional index (PINI), which combines the measurement of two inflammatory proteins (CRP and orosomucoid) and two nutritional-based proteins

*Address correspondence to this author at Food Science and Technology Department, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire; Tel: (225) 07 07 79 03 64; Fax: (225) 27 22 44 21 08;
E-mail: edipagbo@gmail.com

(albumin and prealbumin). However, most studies have assessed malnutrition against anthropometric indices (weight loss, body mass index, measurement of brachial or calf circumference). These measurements are certainly specific to the compartments measured, but they are also not very sensitive [11]. However, in children aged over 5 years, the pathologies have a rather insidious installation and evolution [12]. Thus, the determination of PINI in children is necessary because it could better guide the management of malnutrition.

The objective of this work was to study the impact of food diversification based on sweet potato, soya, and cowpea on PINI in school children in Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1. Study Population

This research was a longitudinal study conducted over one school year (9 months). It was carried out in four zones (Buyo, Gueyo, Meagui, Soubré) of the Nawa region where the "Vision for change" project set up a cocoa rehabilitation program integrating assessment of the nutritional status of school children visiting school canteens.

The children who participated in this study were between 6 and 12 years old, and consent was obtained from the parents. In addition, the children's general health was certified by a health worker (nurse) during a clinical and laboratory examination before the study took place. Pupils whose age was less than 6 years or over 12 years were not considered in this study.

2.2. Determination of the Sample Size

The sample size was calculated using the Leslie Fischer formula below, considering the prevalence of chronic malnutrition in this area [1].

$$n = \frac{Z^2 p(1-p)}{i^2}$$

$$n = \frac{(1.96)^2 \times 0.1836\,(1-0.1836)}{(0.05)^2}$$

n: minimum sample size for obtaining significant results at a fixed risk level Z.

Z: confidence level (the typical value for the 95% confidence level is 1.96).

p: proportion of children suffering from chronic malnutrition, estimated at 18.36% in the area according to N'go *et al*. [1].

Considering a 10% margin of error, the range of the sample size is (207-253). The study, therefore, considered 240 pupils at a rate of 60 pupils per locality.

2.3. Sampling

A standardized questionnaire survey on the sociodemographic data of 240 students from schools in these different localities was carried out.

In addition, blood samples were taken from the students at different times or stages (phases) of the study:

Phase 0: phase before food consumption;

Phase 1: phase after 3months or one-quarter of the experiment;

Phase 2: phase after 6months of the experiment.

2.4. Preparation of Dishes

Over a school calendar year, the students ate different dishes made with rice, sweet potatoes, soybeans, and cowpeas in school canteens. The dishes were prepared according to the following methods:

2.4.1. Rice with Tomato Soup and Fish

Five kilograms of rice were prepared for 30 mins in a cooking pot containing tap water, oil, and onion. After sautéing the onions, garlic, and tomato stew in a little oil, the fish was stirred in with water. The whole thing was left to simmer for 30 to 40 mins, adding cooking salt, pepper, and nutmeg.

2.4.2. Sweet Potato Porridge Enriched with Green Soybeans

The sweet potato porridge enriched with green soybeans was made by soaking 1kg of green soybeans soaked in hot water, then cooking them for 30 mins in water with a little salt. Ten kilograms of sweet potato were peeled and cut up. After mixing the onions and garlic in a little oil, the tomato paste, precooked soybeans, and water were added. The whole thing was simmered for 20 mins; then, the sweet potato was incorporated. After salting and seasoning with pepper and nutmeg, the whole thing was left to cook for another 30 mins.

2.4.3. Sweet Potato Porridge Enriched with White Cowpeas

The sweet potato porridge enriched with white cowpea was prepared by soaking 1kg of white cowpeas in hot water, then cooking them for 45 mins in water with a little salt. Ten kilograms of sweet potato were peeled and cut up. After mixing the onions and garlic in a little oil, the tomato stew, precooked cowpeas, and water were added. The whole was simmered for 20 mins, and then the sweet potato was incorporated. After salting and seasoning with pepper and nutmeg, the whole thing was left to cook for another 30 mins.

2.4.4. Sweet Potato Porridge Enriched with Green Soybeans and White Cowpeas

One kilogram of green soybeans was soaked in hot water, then cooked for 30 mins in water with a little salt. One kilogram of white cowpeas was soaked in hot water, skinned, and then soaked for 45 mins in water with a little salt. Ten kilograms of sweet potato were peeled and cut up. After mixing the onions and garlic in a little oil, the precooked tomato stew, cowpeas and soybeans were added. The whole thing was simmered for 20 to 30 mins, and then the sweet potato was stirred in. After salting and seasoning with pepper and nutmeg, the whole thing was left to cook for another 30 mins.

2.5. Distribution of Dishes

The different menus were taken as lunch in the school canteen two days a week (Monday and Thursday) during the study. Each child received approximately 300 to 500 g of food. In the study, each group consisted of students from different schools. Each group received the same meal, distributed as follows:

- Group 1 (control group): rice with tomato stew and fish (RSP). These are the dishes usually served in canteens;
- Group 2: sweet potato porridge enriched with green soybeans (PDS);
- Group 3: sweet potato porridge enriched with white cowpeas (PDN);
- Group 4: sweet potato porridge enriched with green soybeans and white cowpeas (PDSN).

2.6. Assay of Biochemical Parameters

The assay of the biochemical parameters was carried out using a COBAS c311 analyzer.

2.6.1. C-Reactive Protein

The concentration of CRP was determined by immunoturbidimetry with latex particles sensitized by specific antibodies [13]. This is because CRP reacts in an agglutination reaction with latex particles coated with monoclonal anti-CRP antibodies. The precipitate obtained is measured by turbidimetry at 552 nm [14].

The assay was carried out with a COBAS c311 analyzer using 2 µL of serum to which was added 82 µL of R14 solution associated with 48 µL of diluent (H_2O), then 28 µL of R15 solution combined with 14 µL of diluent (H_2O). The result was obtained 10 mins later.

2.6.2. Orosomucoid

Orosomucoidor α-1-acid glycoprotein constitutes a marker of the inflammatory reaction. It was assayed by immunoturbidimetry [15], using 2 µL of serum to which was added 35 µL of R16 solution combined with 17 µL of diluent (H_2O).

2.6.3. Albumin

Albumin concentrations were determined using a modified bromocresol green binding assay [16]. Albumin at pH 4.3 is sufficiently cationic to combine with bromocresol green (BCG) and form a blue-green-colored complex [17]. The intensity of the blue-green color is directly proportional to the concentration of albumin in the sample. It is measured by the increase in absorbance at 629 nm.

The albumin assay was carried out using a COBAS c311 analyzer using 2 µL of serum, to which was added 100 µL of R4 solution (mono-reagent) and 20 µL of diluent (H_2O). The result was obtained 10 mins later.

2.6.4. Prealbumin

Prealbumin forms a precipitate in the presence of a specific antiserum; this precipitate can be assayed by immunoturbidimetry at 340 nm [18].

The prealbumin assay using a COBAS c311 analyzer uses 2 µL of the serum to which has been added 100 µL of R4 solution (mono-reagent) and 20 µL of diluent (H_2O). The result was obtained after 10 min.

2.6.5. Prognostic Inflammatory and Nutritional Index

Since the plasma concentrations of so-called nutritional proteins are influenced by inflammatory syndrome, Ingenbleek and Carpentier [10] proposed to

correct the fluctuations of these proteins by variations in more specific proteins of an inflammatory syndrome such as CRP and orosomucoid. In 1985, they proposed an index, PINI, which combines the measurement of two inflammatory proteins (CRP and orosomucoid) and two nutrition proteins (albumin and prealbumin), according to the following equation:

$$\text{PINI} = \frac{\text{CRP} \times \text{Orosomucoid}}{\text{Albumin} \times \text{Prealbumin}}$$

This index makes it possible to classify malnourished patients into five groups:

- PINI < 1: patient not infected, not malnourished
- PINI = 1 to 10: patient at low risk
- PINI = 11 to 20: patient at moderate risk
- PINI = 21 to 30: patient at high risk of complications
- PINI > 30: patient at critical risk

2.7. Statistical Analysis Methods

Statistical analysis was performed using GraphPad Prism 5 Demo software, and Student's t-test was used to compare the variances of the means. Relationships between data were assessed using Pearson's chi-squared test. A value of $p < 0.05$ is considered statistically significant. Data are presented as the mean ± standard deviation of the mean.

3. RESULTS

3.1. Average Values of the Biological Parameters of the Study Population

Generally, protein values were within the range of reference values. However, for albumin, students in groups 3 (PDN) and 4 (PDSN) showed a slight increase in mean values compared to group 1, the control (RSP). These values varied from 41.32 ± 0.95 to 42.24 ± 0.95 g/L in group 3 and from 37.07 ± 1.12 to 41.51 ± 1.71 g/L in group 4. On the other hand, in group 1 (control group, RSP), the concentrations varied from 39.79 ± 0.94 to 39.55 ± 0.86 g/L. This increase was significant in phase 1 (3 months after food consumption), with a p-value <0.0001 for group 3 and $p = 0.0003$ for group 4.

In contrast, in group 2 (PDS), there was a non-significant decrease ($p > 0.05$) in the mean albumin value (41.25 ± 0.98 to 38.16 ± 1.17 g/L) compared to group 1 (RSP). In group 2, the mean prealbumin values decreased without significant difference, ranging from 0.15 ± 0.04 to 0.13 ± 0.04 g/L, whereas in groups 3 and 4, the mean prealbumin values remained constant.

The mean CRP value in the different groups decreased from phase 1 for group 1 (2.71 ± 0.64 to 2.06 ± 0.26 mg/L) and group 4 (2.84 ± 0.46 to 2.38 ± 0.36 mg/L) and inphase 2 for groups 2 (3.67 ± 0.99 to 1.98 ± 0.32 mg/L) and 3 (7.89 ± 2.39 to 2.55 ± 0.32 mg/L), compared to group 1.

Regarding the mean value of orosomucoid, it increased in group 3 (0.70 ± 0.04 to 0.74 ± 0.04 g/L) and group 4 (0.67 ± 0.04 to 0.74 ± 0.04 g/L) without significant difference ($p > 0.05$). On the other hand, adecrease in this parameter was observed in group 2 (0.73 ± 0.03 to 0.64 ± 0.05 g/L) without significant difference, compared to group 1.

The mean value of PINI was less than 1 in the whole study. Thus, whatever the group or phase, a progressive decrease in the mean value of PINI was observed: from 0.75 to 0.38 in group 1, from 0.70 to 0.34 in group 2, from 0.91 to 0.45 in group 3, and from 0.83 to 0.39 in group 4 (Table **1**).

3.2. Percentage Reduction in Prognostic Inflammatory and Nutritional Index

The reduction in PINI was around 0.37 in group 1 (RSP), 0.36 in group 2 (PDS), 0.46 in group 3 (PDN), and 0.44 in group 4 (PDSN). The greatest reduction concerned the pupils of group 3 (Figure **1**).

3.3. Distribution of the Study Population According to PINI Values

Whatever the group or phase, more than 80% of this study population were uninfected and not malnourished (PINI <1). Indeed, 6months after the consumption of meals, 90%, 95%, 86.7%, and 90% of the population of groups 1 (RSP), 2 (PDS), 3 (PDN), and 4 (PDSN), respectively, were in this situation. However, a low risk of inflammation in 5% to 20% of children and a moderate risk of inflammation in 1.7% of children have been shown. In fact, 6 months after the consumption of meals, 10%, 5%, 13.3%, and 10% of the population of groups 1 (RSP), 2 (PDS), 3 (PDN), and 4 (PDSN), respectively, had a low risk of inflammation (Table **2**).

Table 1: Mean Values of Protein Parameters and Prognostic Inflammatory and Nutritional Index of the Study Population

Parameter	Phase	Group 1 (n= 60)	Group 2 (n= 60)	P-value[a]	Group 3 (n= 60)	P-value[b]	Group 4 (n= 60)	P-value[c]
Albumin (35-45 g/L)	0	39.79 ± 0.94	41.25 ± 0.98	0.2852	41.32 ± 0.95	0.2557	37.07 ± 1.12	0.0654
	1	34.87 ± 1.33	38.16 ± 1.17	0.0662	41.82 ± 0.92	< 0.0001	40.59 ± 0.73	0.0003
	2	39.55 ± 0.86	38.16 ± 1.37	0.3939	42.24 ± 0.76	0.0213	41.51 ± 0.72	0.0853
Prealbumin (0.20-0.40 g/L)	0	0.14 ± 0.03	0.15 ± 0.04	0.1258	0.14 ± 0.04	0.9863	0.13 ± 0.03	0.1804
	1	0.15 ± 0.03	0.13 ± 0.04	0.0020	0.14 ± 0.04	0.1290	0.14 ± 0.04	0.0936
	2	0.14 ± 0.04	0.13 ± 0.04	0.2100	0.14 ± 0.08	0.8060	0.13 ± 0.04	0.2039
C-reactive protein (< 10 mg/L)	0	3.65 ± 0.69	3.19 ± 0.62	0.6180	4.29 ± 0.90	0.5740	7.89 ± 2.39	0.6972
	1	2.71 ± 0.64	3.67 ± 0.99	0.4173	7.89 ± 2.39	0.4596	2.84 ± 0.46	0.8645
	2	2.06 ± 0.26	1.98 ± 0.32	0.8475	2.55 ± 0.32	0.2394	2.38 ± 0.36	0.4799
Orosomucoid (B: 0.52-1.25 g/L) (G: 0.48-1.29 g/L)	0	0.80 ± 0.03	0.73 ± 0.03	0.1358	0.70 ± 0.04	0.0516	0.67 ± 0.04	0.0142
	1	0.81 ± 0.03	0.71 ± 0.05	0.0249	0.73 ± 0.03	0.0184	0.71 ± 0.04	0.0066
	2	0.80 ± 0.03	0.64 ± 0.05	0.0061	0.74 ± 0.04	0.2481	0.74 ± 0.04	0.2245
PINI	0	0.75 ± 0.17	0.70 ± 0.23	0.8527	0.91 ± 0.22	0.5854	0.83 ± 0.20	0.7675
	1	0.68 ± 0.25	0.51 ± 0.10	0.5501	0.39 ± 0.06	0.2698	0.42 ± 0.08	0.3368
	2	0.38 ± 0.07	0.34 ± 0.08	0.6707	0.45 ± 0.08	0.4589	0.39 ± 0.07	0.8967

a: group 1 vs. group 2; b: group 1 vs. group 3; c: group 1 vs. group 4. The difference is significant for $p<0.05$ (Student's t test).
B:Boy; G: Girl.

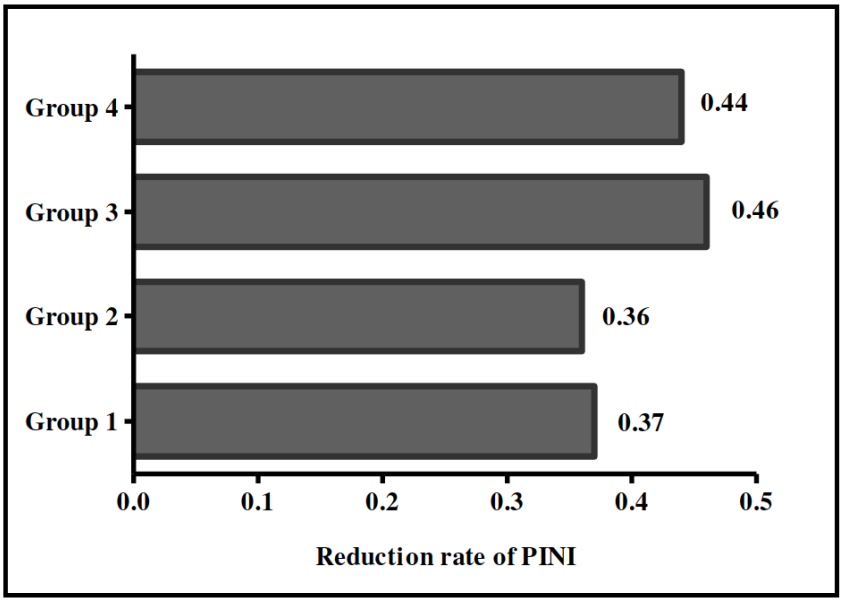

Figure 1: Variation of mean PINI value during the study.

4. DISCUSSION

PINI groups together two inflammatory parameters (CRP and orosomucoid) and two nutritional parameters (albumin and prealbumin) [10]. It is determined in order to simultaneously assess the inflammatory and nutritional status. This index is used to classify patients as uninfected and not malnourished or at low, moderate, high, or critical risk of complications and inflammation [19].

In this study, a gradual decrease in the mean values of PINI was observed in all groups of students over the period in which they consumed the different dishes, with reduction rates between 0.36 and 0.47. This decrease in PINI values agrees with the results of Vehe

Table 2: Distribution of the Study Population According to the Inflammatory Process

Group	Phase	PINI < 1	1 ≤ PINI < 10	11 ≤ PINI < 20	21 ≤ PINI < 30	PINI > 30
1	0	49 (81.7%)	11 (18.3%)	0 (0%)	0 (0%)	0 (0%)
	1	53 (88.3%)	6 (10%)	1 (1.7%)	0 (0%)	0 (0%)
	2	54 (90%)	6 (10%)	0 (0%)	0 (0%)	0 (0%)
2	0	51 (85%)	8 (13.3%)	1 (1.7%)	0 (0%)	0 (0%)
	1	51 (85%)	9 (15%)	0 (0%)	0 (0%)	0 (0%)
	2	57 (95%)	3 (5%)	0 (0%)	0 (0%)	0 (0%)
3	0	48 (80%)	12 (20%)	0 (0%)	0 (0%)	0 (0%)
	1	52 (86.7%)	8 (13.3%)	0 (0%)	0 (0%)	0 (0%)
	2	52 (86.7%)	8 (13.3%)	0 (0%)	0 (0%)	0 (0%)
4	0	48 (80%)	12 (20%)	0 (0%)	0 (0%)	0 (0%)
	1	54 (90%)	6 (10%)	0 (0%)	0 (0%)	0 (0%)
	2	54 (90%)	6 (10%)	0 (0%)	0 (0%)	0 (0%)

et al. [20], who observed a significant decrease in PINI values from the start to the finish of their study. According to them, these low values of PINI (below 1) would be due to a decrease in the concentration of CRP, a slight increase in the concentrations of prealbumin and albumin, and the non-variation of orosomucoid concentration, as also observed in this present study during the different phases. Indeed, during malnutrition, a decrease in nutritional proteins is observed in favor of inflammatory proteins [9]. The slight increase in albumin observed in this study would be due to the consumption of dishes made from sweet potato (*I. batatas*), known for its high protein content [6,21,22], especially in groups 3 (PDN) and 4 (PDSN). The same is true for soybean (*G. max*) [7] and cowpea (*V. unguiculata*), which represent an important source of protein to some populations [8,23]. This result clearly shows the positive impact of dietary diversification on PINI, particularly CRP, thus showing an absence of inflammatory syndrome in the entire study population. Indeed, the nutritional composition of foods is involved in the fight against malnutrition, the biological signs of which are disruption of nutritional and inflammatory proteins. In addition, during malnutrition, the body is faced with two important requirements.

On the one hand, the body must have sufficient resources (proteins) to fight against the causes of malnutrition; on the other, it is necessary to repair the consequences of malnutrition by reconstituting its stock of proteins in order to support the functioning of other organs [24]. However, a low risk of inflammation in 5% to 20% of children and a moderate risk of inflammation in 1.7% of children have been shown. These results can be explained by the infectious tropical environment in which these children live [25]. Indeed, several studies have shown that tropical pathologies, particularly parasitoses (intestinal parasites, *Plasmodium falciparum*, and others), tend to constantly stimulate the immune system [26].

5. CONCLUSION

In this study, a progressive decrease in the mean PINI value was observed in all groups of students during the period they consumed the different dishes. This situation concerned more than 80% of the children, whatever the group or phase, and would be due to a decrease in CRP concentration, a slight increase in the concentrations of prealbumin and albumin, and the non-variation of orosomucoid concentration. The greatest reduction concerned pupils who consumed the meal based on sweet potato porridge enriched with white cowpea. Dietary diversification, particularly through the intake of protein provided by meals based on sweet potato and white cowpea, has a positive impact on PINI and hence on nutritional status.

ETHICAL APPROVAL

This study was authorized by the national research ethics committee (N/Ref: 009//MSHP/CNER-Kp). In addition, the authorities of each village and the school headteachers were contacted and informed before the start of the study in their localities. Their approval was required before the start of the study.

CONSENT

A standardized questionnaire survey focusing in particular on food and hygiene habits and medical history was conducted for each child. Informed, written, and signed consent was obtained from each parent or guardian of the participating students after discussion of the purpose and benefits of the study and from each manager of each institution involved in the study. Participants were informed of their volunteering and of the right to leave the survey at any time. The confidentiality of the information of study participants was also ensured. Only anonymized data were used for statistical analysis.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

SUPPLEMENTAL MATERIALS

The supplemental materials can be downloaded from the journal website along with the article.

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